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(54) Title: TREATMENT AND PREVENTION OF HELICOBACTER INFECTION

(57) Abstract

An antigenic preparation for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, comprises the catalase enzyme of *Helicobacter* bacteria, particularly the catalase enzyme of *H. pylori* or *H. felis*, or an immunogenic fragment thereof.

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TREATMENT AND PREVENTION OF HELICOBACTER INFECTION

FIELD OF THE INVENTION

This invention relates to protective *Helicobacter* antigens, especially *H. pylori* antigens, and to the use of these antigens for the treatment and prevention of gastroduodenal disease associated with *H. pylori* infection in humans.

BACKGROUND OF THE INVENTION

Helicobacter pylori is a bacterium that infects the stomach lining (or gastric mucosa) of perhaps half the world's population. Spiral organisms were first microscopically observed in human gastric mucosa in 1906. However, *H. pylori* was not successfully cultured until 1982. Infection with the organism is usually chronic, and results in continuing inflammation of the gastric mucosa. The infection is often asymptomatic. However, in association with other cofactors, a proportion of infected people go on to develop sequelae including peptic ulceration of the stomach or duodenum, gastric adenocarcinomas and gastric lymphomas. Peptic ulcer treatment studies have shown that cure of *H. pylori* infection is associated with a dramatic reduction in the relapse rate of this usually chronic disease. Long term infection with *H. pylori* leads to the development of chronic atrophic gastritis, which has long been recognised as a precursor lesion in the development of gastric cancer. Thus a number of studies have now linked preceding *H. pylori* infection with an increased risk of developing gastric cancer. Therefore eradication of current infection and prevention of new infection with this organism has the potential to significantly reduce the incidence of diseases that result in considerable morbidity and mortality[1,2].

Infection with *H. pylori* is difficult to treat. Current experimental therapies for treating the infection have problems with efficacy and significant levels of adverse effects. There are no prophylactic measures available. A solution to

both the prevention and treatment of *H. pylori* infection would be the development of an immunogenic preparation that, as an immunotherapeutic, treated established infections, and as a vaccine, prevented the establishment of new or recurrent infections. Such a preparation would need to induce effective immune responses to protective antigens, while avoiding inducing responses to self antigens or other potentially harmful immune responses. This may be achieved by identifying the specific protective component or components and formulating immunotherapeutic or vaccine preparations including these component(s).

The identification of such protective components of an organism, is often accomplished through the use of an animal model of the infection. *H. pylori* does not naturally infect laboratory animals. However, an animal model of human *H. pylori* infection has been developed using a closely related organism, *H. felis*, and specific pathogen free (SPF) mice[3]. These organisms are able to colonise the gastric mucosa of SPF mice, where they establish a chronic infection with many of the features of *H. pylori* infection in humans. *H. felis* infection in the mice induces a chronic gastritis and a raised immune response. As in the human case, this response is not effective in curing the infection.

This model has been used to demonstrate that oral treatment of *H. felis* infected mice with a preparation containing disrupted *H. pylori* cells and cholera toxin as a mucosal adjuvant, can cure a significant portion of infected mice[4]. This effect is likely to be mediated through an immune response to a cross-reactive antigen possessed by each of the closely related species.

In working by the inventors leading to the present invention, these cross-reactive antigens were recognised by performing a Western blot using *H. pylori* disrupted cells as the antigen, and probing the blot with serum from mice immunised with *H. felis* and cholera toxin adjuvant. Sections of membrane containing proteins recognised as cross-reactive were removed from the

membrane, the proteins bound to them were eluted, and their N-terminal amino acid sequence determined by microsequencing.

The N-terminal amino acid sequence of one of the two proteins that successfully yielded sequence data closely matched the previously published sequence of the microbial enzyme, urease[5]. This enzyme has already been shown to be a protective antigen when used in a vaccine to prevent infection.

The N-terminal amino acid sequence of the other protein closely matched the previously published N-terminal sequence of the microbial enzyme, catalase[6]. This enzyme has not previously been shown to be a protective antigen of *H. pylori.*

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an antigenic preparation for use in the treatment or prevention of *Helicobacter* infection, which comprises an at least partially purified preparation of the catalase of *Helicobacter* bacteria.

The term "at least partially purified" as used herein denotes a preparation in which the catalase content is greater, preferably at least 30% and more preferably at least 50% greater, than the catalase content of a whole cell sonicate of *Helicobacter* bacteria. Preferably, the preparation is one in which the catalase is "substantially pure", that is one in which the catalase content is at least 80%, more preferably at least 90%, of the total *Helicobacter* antigens in the preparation.

It is to be understood that the present invention extends not only to an antigenic preparation comprising the catalase of *Helicobacter* bacteria, but also to antigenic preparations comprising immunogenic fragments of this catalase, that is catalase fragments which are capable of eliciting a specific protective immune response in a mammalian host. Such immunogenic fragments may also be

recognised by *Helicobacter*-specific antibodies, particularly monoclonal antibodies which have a protective or therapeutic effect in relation to *Helicobacter* infection or polyclonal antibodies contained in immune sera from mammalian hosts which have been vaccinated against *Helicobacter* infection.

In another aspect, the present invention provides a vaccine composition for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises an immunologically effective amount of an antigenic preparation as broadly described above, optionally in association with an adjuvant, together with one or more pharmaceutically acceptable carriers and/or diluents.

In yet another aspect, the present invention provides a method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation as broadly described above, optionally in association with an adjuvant.

In a related aspect, this invention provides the use of a vaccine composition comprising an immunologically effective amount of an antigenic preparation as broadly described above, optionally in association with an adjuvant, for the treatment or prevention of *Helicobacter* infection in a mammalian host.

In yet another aspect, the invention provides the use of an antigenic preparation as broadly described above, optionally in association with an adjuvant, in the manufacture of a vaccine composition for the treatment or prevention of *Helicobacter* infection in a mammalian host.

Preferably, but not essentially, the antigenic preparation of this invention is orally administered to the host, and is administered in association with a

mucosal adjuvant. However, the invention also extends to parenteral administration of this antigenic preparation.

The present invention also extends to delivery of the antigenic preparation of this invention to the host using a vector expressing the catalase of *Helicobacter* bacteria, or an immunogenic fragment thereof. Accordingly, in a further aspect this invention provides a preparation for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises a vector expressing the catalase of *Helicobacter* bacteria or an immunogenic fragment thereof.

In this aspect, the invention extends to a method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises administration to said host of a vector expressing the catalase of *Helicobacter* bacteria or an immunogenic fragment thereof.

Further, the invention extends to the use of a vector expressing the catalase of *Helicobacter* bacteria or an immunogenic fragment thereof, for the treatment or prevention of *Helicobacter* infection in a mammalian host.

The present invention also extends to an antibody, which may be either a monoclonal or polyclonal antibody, specific for an antigenic preparation as broadly described above.

In this aspect, the invention further provides a method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises passive immunisation of said host by administration of an immunologically effective amount of an antibody, particularly a monoclonal antibody, specific for an antigenic preparation as broadly described above.

By use of the term "immunologically effective amount" herein in the context of treatment of *Helicobacter* infection, it is meant that the administration of that amount to an individual infected host, either in a single dose or as part of a

series, that is effective for treatment of *Helicobacter* infection. By the use of the term "immunologically effective amount" herein in the context of prevention of *Helicobacter* infection, it is meant that the administration of that amount to an individual host, either in a single dose or as part of a series, that is effective to delay, inhibit or prevent *Helicobacter* infection. The effective amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", is to be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

DETAILED DESCRIPTION OF THE INVENTION

Preferably, the antigenic preparation of this invention comprises a preparation of the catalase of *H. pylori* or *H. felis*, most preferably *H. pylori* catalase. Preferably also, this antigenic preparation is used in a vaccine composition for oral administration which includes a mucosal adjuvant.

In a particularly preferred aspect of this invention, an oral vaccine composition comprising an antigenic preparation of at least partially purified *H. pylori* catalase in association with a mucosal adjuvant is used for the treatment or prevention of *H. pylori* infection in a human host.

The mucosal adjuvant which is optionally, and preferably, administered with the at least partially purified *Helicobacter* catalase preparation to the infected host is preferably cholera toxin. Mucosal adjuvants other than cholera toxin which may

be used in accordance with the present invention include non-toxic derivatives of cholera toxin, such as the B sub-unit (CTB), chemically modified cholera toxin, or related proteins produced by modification of the cholera toxin amino acid sequence. These may be added to, or conjugated with, the *Helicobacter* catalase preparation. The same techniques can be applied to other molecules with mucosal adjuvant or delivery properties such as *Escherichia coli* heat labile toxin. Other compounds with mucosal adjuvant or delivery activity may be used such as bile; polycations such as DEAE-dextran and polyornithine; detergents such as sodium dodecyl benzene sulphate; lipid-conjugated materials; antibiotics such as streptomycin; vitamin A; and other compounds that alter the structural or functional integrity of mucosal surfaces. Other mucosally active compounds include derivatives of microbial structures such as MDP; acridine and cimetidine.

The *Helicobacter* catalase preparation may be delivered in accordance with this invention in ISCOMS (immune stimulating complexes), ISCOMS containing CTB, liposomes or encapsulated in compounds such as acrylates or poly(DL-lactide-co-glycoside) to form microspheres of a size suited to adsorption by M cells. Alternatively, micro or nanoparticles may be covalently attached to molecules such as vitamin B12 which have specific gut receptors. The *Helicobacter* catalase preparation may also be incorporated into oily emulsions and delivered orally. An extensive though not exhaustive list of adjuvants can be found in Cox and Coulter[7].

Other adjuvants, as well as conventional pharmaceutically acceptable carriers, excipients, buffers or diluents, may also be included in the prophylactic or therapeutic vaccine composition of this invention. The vaccine composition may, for example, be formulated in enteric coated gelatine capsules including sodium bicarbonate buffers together with the *Helicobacter* catalase preparation and cholera toxin mucosal adjuvant.

The formulation of such therapeutic compositions is well known to persons skilled in this field. Suitable pharmaceutically acceptable carriers and/or diluents

include any and all conventional solvents, dispersion media, fillers, solid carriers, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art, and it is described, by way of example, in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, Pennsylvania, USA. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the pharmaceutical compositions of the present invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

As an alternative to the delivery of the *Helicobacter* catalase preparation in the form of a therapeutic or prophylactic oral vaccine composition, the catalase or an immunogenic fragment thereof may be delivered to the host using a live vaccine vector, in particular using live recombinant bacteria, viruses or other live agents, containing the genetic material necessary for the expression of the catalase or immunogenic fragment as a foreign antigen. Particularly, bacteria that colonise the gastrointestinal tract, such as *Salmonella, Yersinia, Vibrio, Escherichia* and BCG have been developed as vaccine vectors, and these and other examples are discussed by Holmgren *et al.*[8] and McGhee *et al.*[9].

The *Helicobacter* catalase preparation of the present invention may be administered as the sole active immunogen in a vaccine composition or expressed by a live vector. Alternatively, however, the vaccine composition may include or the live vector may express other active immunogens, including other *Helicobacter* antigens such as urease or the lipopolysaccharide (LPS) of *Helicobacter* bacteria (see International Patent Application No. PCT/AU95/00077), as well as immunologically active antigens against other pathogenic species.

It is especially advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the human subjects to be treated; each unit containing a predetermined quantity of active

ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier and/or diluent. The specifications for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active ingredient and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active ingredient for the particular treatment.

Data obtained from Western blots mentioned above, show that *H. pylori* catalase is recognised by the serum of mice vaccinated with an *H. felis* antigen preparation (plus cholera toxin adjuvant). These mice can be shown to be protected against *H. felis* infection. This data indicates the use of *H. pylori* catalase as a protective antigen in human *H. pylori* infection, and purified or recombinant catalase may be used as an antigenic component of a therapeutic or prophylactic vaccine, either on its own, or in combination with other antigens, carriers, adjuvants, delivery vehicles or excipients.

Further details of the present invention are set out, by way of illustration only, in the following Examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention, and should not be understood in any way as a restriction on the broad description of the invention as set out above.

EXAMPLE 1

A. METHODS

Sonicated *H. pylori* cells were separated in a 12% discontinuous (i.e. homogeneous) SDS-PAGE gel under denaturing conditions using a Mini-Protean II apparatus (Bio-Rad). Proteins were transferred from the gel to ProBlott (Applied Biosciences PVDF-polyvinylidene difluoride) membrane using CAPS buffer (3-(cyclohexylamino)-1-proanesulphonic acid buffer) in a Mini transblot system (Bio-Rad).

Strips were removed from the ends of the PVDF and reacted with immune sera from mice vaccinated with *H. felis* plus cholera toxin and traced with an HRP labelled anti-mouse sera and developed using 4-chloro-1-naphthol as per standard Western blot methods. The remainder of the PVDF was stained with Coomassie blue (Bio-Rad) to visualise the protein bands. Six proteins recognised by the immune sera were selected and the corresponding Coomassie stained bands on the PVDF were carefully excised for sequencing.

The six excised bands of PVDF were cut into small pieces (approx. 0.5 cm long) and placed into the reaction cartridge of an Applied Biosystems Model 476A Protein Sequencer System. All chemistry, HPLC separations, data quantitation and protein sequencing reporting is automatically carried out in this system.

B. RESULTS

Four samples gave no signal in the Protein Sequencer System. Two samples gave clear amino acid sequence data: sample 5, an approximately 53 kD protein (±10%), and sample 3, an approximately 66 kD protein (±10%). This data is shown below.

(i) Sample 3:

D D N

M K K I V F K E Y V

A P

Note: the first three cycles gave equivocal results.

The sequence data of sample 3 corresponds closely, but not exactly, with the previously published N-terminal sequence for the enzyme urease[5]. This enzyme has been shown to be a protective antigen in studies using the *H. felis*/ mouse model.

(ii) Sample 5:

M V N K D V K Q T T A F G T P

The sequence data of sample 5 corresponds closely, with one difference, to the previously published N-terminal sequence of the enzyme catalase[6]. This enzyme has not previously been shown to be a protective antigen however the fact that the enzyme is recognised by the immune serum of mice vaccinated with an *H. felis* antigen preparation to protect against *H. felis* infection, combined with the fact that mice vaccinated with an *H. pylori* antigen preparation are protected against *H. felis* infection, indicates the *H. pylori* catalase as a protective antigen in *H. pylori* infection in humans.

EXAMPLE 2

1. PURIFICATION OF *H. PYLORI* catalase[10]

Approximately 60 plates (CSA) of *H. pylori* (clinical strain 921023) were grown in 10% CO_2 at 37°C for 48 hours. All following steps until loading on the column were undertaken on ice. The *H. pylori* cells were harvested in 0.1 M sodium phosphate buffer pH 7.2 and the suspension spun down gently and resuspended in no more than 5 mL of 0.1 M sodium phosphate buffer. The suspension was then sonicated at 6 kHz 40% duty cycle for 5 minutes. Following this, the sonicate was spun for 5 minutes at 10,000 g, the supernatant collected and passed through a 0.22 μm filter into a sterile container.

The filtrate was loaded onto a K26/100 gel filtration column of Sephacryl S-300 HR and eluted using sodium phosphate buffer at a flow rate of 1.0 mL min^{-1}. The eluate was collected into fractions (100 drops/fraction) and those containing catalase identified by testing for catalase activity (1 drop of the fraction placed in H_2O_2 diluted 1:10 in distilled water and examined for bubbling). Fractions containing the strongest catalase activity were pooled then diluted 1:10

in 0.01M sodium phosphate (filtered). The fractions were then run through a MEMSEP 1000 cm ion exchange capsule. 100 mL of the 0.01 M sodium phosphate buffer was then run through the ion exchange capsule to remove any excess proteins. 1 M NaCl in 0.1 M sodium phosphate buffer was run through the ion exchange capsule to elute out the catalase. Catalase positive fractions were identified by their strong yellow colour and confirmed b testing for a bubbling reaction in H_2O_2.

The catalase positive fractions were stored at 4°C and protected from light. Each fraction was tested for protein concentration using the Bio-Rad DC protein assay, and selected for immunising mice if it contained over 1.5 mg/mL of protein. Prior to immunising mice the purified catalase was checked for contaminants using 12% SDS-PAGE. Proteins were visualised by staining with Coomassie Blue, which indicated that the catalase preparation was at least 95% pure. Image analysis indicated that the catalase's molecular weight was 52-53 kDa. The purified catalase was also strongly recognised by a catalase monoclonal antibody.

2. IMMUNISATION WITH *H. PYLORI* CATALASE.

Sufficient purified catalase for immunising 10 mice was obtained and pooled. Mice were given 0.2 mg purified catalase +10 μg cholera toxin (CT) 4 times on days 0, 7, 14 and 21. Control groups were given cholera toxin alone or PBS buffer alone. The dose size was 150 μl for all groups. On the day of each immunising dose, the catalase was checked for activity using $_2$ and for any signs of degradation using SDS-PAGE and Coomassie Blue staining. No signs of declining activity or any degradation was observed throughout the immunisation course. Three weeks after the last immunising dose all groups were challenged twice with ~10^8 *H. felis*. Three weeks later mice were euthanased and samples (sera, saliva, bile and the stomach - half for histology and half the antrum for the direct urease test) were collected.

Experiment Outline

TIME (days)	CATALASE CT (10 Mice)	CT ALONE (10 mice)	PBS ALONE (10 mice)
0	Cat + CT	CT alone	PBS alone
7	Cat + CT	CT alone	PBS alone
14	CAT + CT	CT alone	PBS alone
21	Cat + CT	CT alone	PBS alone
42	*H. felis* Challenge	*H. felis* Challenge	*H. felis* Challenge
44	*H. felis* Challenge	*H. felis* Challenge	*H. felis* Challenge
65	Collect 10	Collect 10	Collect 10

3. RESULTS

Urease

POSITIVE UREASE RESULT (%)		
Catalase + CT (10)	CT alone (10)	PBS alone (10)
0/10 (0)	7/10 (70)	10/10 (100)

Western Blotting

Western blots of sera from mice showed strong recognition of *H. pylori* catalase by the immunised mice, whereas mice from the other groups showed weak or absent recognition.

Persons skilled in this art will appreciate that variations and modifications may be made to the invention as broadly described herein, other than those specifically described without departing from the spirit and scope of the invention. It is to be understood that this invention extends to include all such variations and modifications.

REFERENCES

1. *Helicobacter pylori* Biology and Clinical Practice (1993). Edited by C.Stewart Goodwin and Bryan W.Worsley. Published by CRC Press.

2. Halter, F., Hurlimann, S. and Inauen, W. (1992). Pathophysiology and clinical relevance of *Helicobacter pylori*. *The Yale Journal of Biology and Medicine,* **65**:625-638.

3. Lee, A., Fox, J.G., Otto, G. and Murphy, J. (1990). A small animal model of human *Helicobacter pylori* active chronic gastritis. *Gastroenterology*, **99**:1316-1323.

4. Doidge, C.G., Gust, I., Lee, A., Buck, F., Hazel, S. and Mane, U. (1994). Therapeutic immunisation against *Helicobacter pylori* - The first evidence. *Lancet* **343**(i):914-915.

5. Clayton, C.L., Pallen, M.J., Kleanthous, H., Wren, B.W. and Tabaqchali, S. (1990). Nucleotide sequence of two genes from *Helicobacter pylori* encoding for urease subunits. *Nucleic Acid Res.*, **18(2)**:362

6. Westblom, T.U., Phadnis, S., Langenberg, W., Yoneda, K., Madan, E. and Midkiff, B.R. (1992). Catalase negative mutants of *Helicobacter pylori*. *European Journal of Clinical Microbiology and Infectious Diseases,* **11**:522-526.

7. Cox, J. and Coulter, A. (1992). Advances in adjuvant technology and application. *In* Animal Parasite Control Using Biotechnology. Edited by W.K.Yong. Published by CRC Press.

8. Holmgren, J., Czerkinsky, C., Lycke, N. and Svennerholm, A-M. (1992). Mucosal Immunity : Implications for Vaccine Development. *Immunobiol.* **184**:157-179.

9. McGhee, J.R., Mestecky, J., Dertzbaugh, M.T., Eldridge, J.H., Hirasawa, M. and Kiyono, H. (1992). The mucosal immune system : from fundamental concepts to vaccine development. *Vaccine* **10**(2):75-88.

10. Hazel, S.L., Evans Jr., D.J. and Graham, D.Y (1991). *Helicobacter pylori* catalase. *J. Gen. Microbiol.* **137**:57-61.

CLAIMS:

1. An antigenic preparation for use in the treatment or prevention of *Helicobacter* infection, which comprises the catalase of *Helicobacter* bacteria, or an immunogenic fragment thereof.

2. An antigenic preparation according to claim 1, which comprises the catalase of *H. pylori* or *H. felis*, or an immunogenic fragment thereof.

3. An antigenic preparation according to claim 1 or claim 2, which comprises an at least partially purified catalase preparation.

4. An antigen preparation according to any one of claims 1 to 3, further comprising at least one additional active immunogen.

5. An antigen preparation according to claim 4, wherein the additional immunogen(s) comprise at least one other *Helicobacter* antigen.

6. An antigen preparation according to claim 5, wherein said other *Helicobacter* antigen is selected from *Helicobacter* urease and *Helicobacter* lipopolysaccharide.

7. A vaccine composition for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises an immunologically effective amount of an antigenic preparation according to any one of claims 1 to 6, together with one or more pharmaceutically acceptable carriers and/or diluents.

8. A vaccine composition according to claim 7, further comprising an adjuvant.

9. A vaccine composition according to claim 8, wherein the adjuvant is a mucosal adjuvant.

10. A method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation according to any one of claims 1 to 6.

11. A method according to claim 10, wherein said antigenic preparation is administered in association with an adjuvant.

12. A method according to claim 11, wherein said adjuvant is a mucosal adjuvant.

13. A method according to any one of claims 10 to 12, wherein said antigenic preparation is orally administered to said host.

14. A method according to any one of claims 10 to 12, wherein said antigenic preparation is parenterally administered to said host.

15. A method according to any one of claims 10 to 14, wherein said host is a human.

16. Use of an immunologically effective amount of an antigenic preparation according to any one of claims 1 to 6, for the treatment or prevention of *Helicobacter* infection in a mammalian host.

17. Use according to claim 16 wherein said antigenic preparation is administered in association with an adjuvant.

18. Use according to claim 17, wherein said adjuvant is a mucosal adjuvant.

19. Use according to any one of claims 16 to 18, wherein said antigenic preparation is orally administered to said host.

20. Use according to any one of claims 16 to 18, wherein said antigenic preparation is parenterally administered to said host.

21. Use according to any one of claims 16 to 20, wherein said host is a human.

22. Use of an antigenic preparation according to any one of claims 1 to 6, optionally in association with an adjuvant, in the manufacture of a vaccine composition for the treatment or prevention of *Helicobacter* infection in a mammalian host.

23. A preparation for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises a vector expressing the catalase of *Helicobacter* bacteria or an immunogenic fragment thereof.

24. A preparation according to claim 23, wherein said vector is a bacterium that colonises the gastrointestinal tract of the mammalian host.

25. A preparation according to claim 24, wherein said vector is a *Salmonella, Yersinia, Vibrio, Escherichia* or BCG bacterium.

26. A method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises administration to said host of a preparation according to any one of claims 23 to 25.

27. Use of a preparation according to any one of claims 23 to 25, for the treatment or prevention of *Helicobacter* infection in a mammalian host.

28. An antibody specific for an antigen preparation according to any one of claims 1 to 6.

29. An antibody according to claim 28, which is a monoclonal antibody.

30. A vaccine composition for use in the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises an antibody according to claim 28 or claim 29, together with one or more pharmaceutically acceptable carriers and/or diluents.

31. A method for the treatment or prevention of *Helicobacter* infection in a mammalian host, which comprises passive immunization of said host by administration of an immunologically of an antibody according to claim 28 or claim 29.

32. Use of an immunologically of an antibody according to claim 28 or claim 29 for the treatment or prevention of *Helicobacter* infection in a mammalian host.

33. Use of an antibody according to claim 28 or claim 29, in the manufacture of a vaccine composition for the treatment or prevention of *Helicobacter* infection in a mammalian host.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 95/00335

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[6] A61K 39/02, A61K 39/40, C07K 16/40, C07K 16/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 39/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
WPAT: A61K/IC, C12N/IC. HELICOBACTER: ANTIGEN: ANTIBODY
BIOTECH: HELICOBACTER. CAMPYLOBACTER PYLORI, ANTIBODY, ANTIGEN
CASM: HELICOBACTER: CATALASE:
JAPIO: A61K/IC, C12N, HELICOBIACTER: ANTIGEN: ANTIBODY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO 93/16723 (VANDERBILT UNIVERSITY) 2 September 1993	1-33
A	AU 55619/94 (FONDATION POURLA RECHERCHE DES MALADIES GASTRO-INTESTINALES: GAS TROFONDS MANDATRIA FIDUCIAIRE SA) 11 May 1994	1-33
A	WO 94/06474 (GALAGEN INC.) 31 March 1994	1-33
A	WO 93/20843 (CZINN S. J. and NEDRUD J. G.) 28 October 1993	1-33

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 1995	17 AUGUST 1995
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA	Authorized officer S. CHANDRA

INTERNATIONAL SEARCH REPORT

Information on patent family membe.

International application No.
PCT/AU 95/00335

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	9316723	AU	37282/93	EP	629132
AU	9455619	EP	625053	WO	9409823
WO	9406474				
WO	9320843	AU	39770/93	EP	590138

END OF ANNEX